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EXAMINER

STRZELECKA, TERESA E

ART UNIT PAPER NUMBER

1637

DATE MAILED: 11/01/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/920,571

Applicant(s)

LASKEN ET AL.

Examiner

Teresa E. Strzelecka

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 15 August 2005.
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) See Continuation Sheet is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1,5-9,14,20,22-25,27,29,31-33,35-39,41,42,44-49,51-53,55-59,69 and 70 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____.

- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____.
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 1,5-9,14,20,22-25,27,29,31-33,35-39,41,42,44-49,51-53,55-59,69 and 70.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114.

Applicant's submission filed on August 15, 2005 has been entered.

2. Claims 1, 5-9, 11-15, 20-25, 27, 29, 31-33, 35-39, 41, 42, 44-49, 51-53 and 55-59 were previously pending. Applicants amended claims 1 and 53, cancelled claims 11-13, 15 and 21 and added new claims 69 and 70. Claims 1, 5-9, 14, 20, 22-25, 27, 29, 31-33, 35-39, 41, 42, 44-49, 51-53, 55-59, 69 and 70 are pending and will be examined.

3. Applicants' claim cancellations overcame the following rejections: rejection of claims 11, 13, 15 and 21 under 35 U.S.C. 103(a) over Lizardi, Landers et al. and Eckstein et al. and rejection of claim 12 under 35 U.S.C. 103(a) over Lizardi, Landers et al. and Eckstein et al. in view of Rothberg et al. Rejection of claims 36 and 37 under 35 U.S.C. 103(a) over Lizardi, Landers et al. and Eckstein et al. in view of Rothberg et al. is withdrawn as it duplicates the rejection of claims 36 and 37 already present over Lizardi, Landers et al., Navarro et al. and Eckstein et al. All other rejections are maintained in a re-phrased form. Applicants' arguments are mostly moot as they refer to previously presented rejections, but they will be addressed to the degree that they pertain to the current rejections.

Response to Arguments

4. Applicant's arguments filed August 15, 2005 have been fully considered but they are not persuasive. Only the pending rejections are addressed.

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A) Regarding the rejection of claims 1, 5-9, 20, 22-25, 27, 29, 31, 33, 35, 38, 39, 41, 44-49, 51-53, 55 and 56 under 35 U.S.C. 103(a) over Lizardi, Landers et al. and Eckstein et al., Applicants argue the following:

a) The references do not disclose or suggest use of random primers in rolling circle replication, since there is no apparent motivation in any of the publications to substitute the random primers of Landers et al. for the defined primers of Lizardi. Landers et al. disclose amplification of linear DNAs using random primers, but they do not provide suggestions for using random primers on circular templates. Applicants further argue that PCR amplification of Landers et al. is “fundamentally different from the rolling circle replication of Lizardi” since rolling circle amplification uses diagnostic probe with a defined primer sequence, and the amplification is isothermal.

b) The references do not disclose or suggest multiple primers in rolling circle replication. Applicants argue that while Lizardi does disclose using multiple primers together in rolling circle replication, Lizardi does not “specifically disclose and certainly does not suggest the use of multiple rolling circle replication primers nor formation of multiple tandem sequence DNA products from multiple primings of a single amplification target circle”.

B) Regarding the rejection of claims 14, 57 and 58 under 35 U.S.C. 103(a) over Lizardi, Landers et al. and Eckstein et al. in view of Navarro et al., Applicants argue that since Lizardi, Landers et al. and Eckstein et al. do not anticipate claim 1, the combination of these references with Navarro et al. fails to disclose, suggest or provide motivation for using random primers in the rolling circle amplification of Lizardi, and fails to suggests claims 14, 57 and 58.

C) Regarding the rejection of claims 32, 42 and 59 under 35 U.S.C. 103(a) over Lizardi, Landers et al. and Eckstein et al. in view of Skerra et al., Applicants argue that since Lizardi,

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Landers et al. and Eckstein et al. do not anticipate claim 1, the combination of these references with Skerra et al. fails to disclose, suggest or provide motivation for using random primers in the rolling circle amplification of Lizardi, and fails to suggest claims 32, 42 and 59.

Regarding A), there are two main issues that Applicants discuss: motivation to use random primers in rolling circle amplification and use of multiple primers simultaneously. As to the first issue, even though Lizardi does not teach or suggest random primers, Landers et al. teach using such primers to amplify unknown sequences, which is a sufficient motivation to use such primers. Applicants argue that Landers et al. teach a “fundamentally different amplification” from the one of Lizardi. However, in the case of Lizardi, a primer (or a set of primers, as will be discussed below), is extended while hybridized to a circular target by a DNA polymerase. In Landers et al., a random primer is extended by a DNA polymerase while hybridized to a target, which, in case it is a YAC, is circular. The fundamental process in each case is the same, i.e., primer extension in the presence of a target, as are the polymerase which are used. The fact that the temperature is cycled or not is not relevant to the fundamental principle of the extension process, e.g., the same product will be produced by a primer extension on a target whether the amplification is at constant temperature or thermally cycled. Further, Applicants did not provide any evidence that extension of random primers requires significantly different conditions from extension of primers of known sequence on a circular target. Finally, the restated rejection includes the reference of Navarro et al., which specifically teaches amplification of single-stranded RNA circles using multiple random primers. Therefore, this reference provides evidence that multiple random primers are successfully used in amplification of single-stranded circular nucleic acids, and motivation to use such primers, namely, to amplify unknown sequences.

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As to the issue of multiple priming of a single target circle, Lizardi discloses the following: The rolling circle amplification (RCA) involves hybridization (= contacting) of a primer to single-stranded amplification target circles (ATC) (col. 9, lines 25-29) followed by amplification using strand-displacing DNA polymerase, resulting in a DNA molecule with multiple repeats of the ATC, usually referred to as tandem sequences DNA (TS-DNA) (column 19, lines 20-31). In one embodiment of the amplification, strand displacement cascade amplification, (SDCA), secondary and tertiary primers are used, with sequences complementary to the ATC, with secondary and tertiary primers not being complementary to the original primer or to each other (col. 25, lines 36-49). The SDCA can be performed simultaneously with RCA, resulting in exponential amplification (col. 25, lines 50-57; col. 28, lines 8-18). Therefore, since initial primers and the secondary and tertiary primers anneal to different parts of ATCs and are present simultaneously in the reaction mixture, and the reaction mixture contains a DNA polymerase, it is inherent in the process that multiple extension products will be produced from each of target circles. Therefore, Lizardi does inherently disclose multiply primed amplification of target circles and production of multiple extension products. Applicants did not provide any evidence of why the secondary and tertiary primers, while present in the rolling circle amplification mix together with the original RCA primer would fail to anneal to the target circles and result in a production of multiple extension products, since the polymerases claimed by Applicants and Lizardi are the same.

The rejection is maintained in a restated form.

Regarding B), the reference of Navarro et al. was discussed above.

The rejection is maintained in a restated form.

Regarding C), rejections of claims 32, 42 and 59, the arguments regarding rejections of claims 1, 31 and 38 were addressed above.

The rejection is maintained in a restated form.

Claim Interpretation

5. Applicants defined the term "random primers" on page 13, lines 20-26: "... As used herein, the term "random" means that said oligonucleotide primers (P1) have nucleotide sequences unrelated to the nucleotide sequences of the amplification target circle (ATC) that acts as template for amplification. The result of such a random relationship is that the locations on the ATC at which said random primers hybridize will also be random."

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

7. Claims 1, 5-9, 14, 20, 22-25, 27, 29, 31, 33, 35-38, 39, 41, 44-49, 51-53, 55-58, 69 and 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lizardi (U.S. Patent No. 5,854,033; cited in the previous office action), Landers et al. (U.S. Patent No. 6,703,228; cited in the previous office action), Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action) and Eckstein et al. (Trends in Bioch. Sci., vol. 14(3), pp. 97-100, 1989; cited in the previous office action).

A) Regarding claim 1, Lizardi teaches a method of amplification comprising contacting multiple single stranded non-circular random oligonucleotide primers (P1), one or more single stranded amplification target circles (ATCs), a DNA polymerase and multiple deoxynucleoside triphosphates (dNTPs), under conditions promoting said contacting, wherein each ATC hybridizes to a plurality of said P1 primers, wherein said conditions promote rolling circle replication of said

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amplification target circle by extension of the P1 primers to form multiple tandem sequence DNA (TS-DNA) products and wherein at least one such dNTP renders the TS-DNA resistant to nuclease activity following incorporation thereinto (Lizardi teaches amplification of circular DNA molecule by a rolling circle method. The rolling circle amplification (RCA) involves hybridization (= contacting) of a primer (= P1) to amplification target circles (ATC) followed by amplification using strand-displacing DNA polymerase, resulting in a DNA molecule with multiple repeats of the ATC, usually referred to as tandem sequences DNA (TS-DNA) (column 19, lines 20-31). Lizardi teaches ATC being a circular, single-stranded DNA molecule, (col. 9, lines 25-29). In one embodiment of the amplification, strand displacement cascade amplification, (SDCA), secondary and tertiary primers are used, with sequences complementary to the ATC, with secondary and tertiary primers not being complementary to the initial primer or to each other (col. 25, lines 36-49). The SDCA can be performed simultaneously with RCA, resulting in exponential amplification (col. 25, lines 50-57; col. 28, lines 8-18). Therefore, since initial primers and the secondary and tertiary primers anneal to different parts of ATCs and are present simultaneously in the reaction mixture, Lizardi teaches the limitation of multiple P1 primers. Lizardi teaches dNTPs (col. 36, lines 50, 51).)

Regarding claims 5-7, Lizardi teaches primers from 10 to 35 nucleotides long (col. 10, line 14), therefore anticipating the limitations of the primers being 2 to 50, 2 to 35 or 2 to 10 nucleotides in length.

Regarding claim 20, Lizardi teaches ATC being a circular, single-stranded DNA molecule, containing between 40 to 1,000 nucleotides (col. 9, lines 25-29), anticipating the limitation of ATC being no larger than 10,000 nucleotides in size.

Regarding claims 22 and 23, Lizardi teaches ATC being a circular, single-stranded DNA molecule, containing between 40 to 1,000 nucleotides (col. 9, lines 25-29), anticipating the

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limitations of ATC being no larger than about 1,000 nucleotides and no larger than about 100 nucleotides in size.

Regarding claim 24, Lizardi teaches that ATC is derived from a single-stranded bacteriophage (col. 35, lines 50-59).

Regarding claim 27, Lizardi teaches that radioactive nucleotides are used in the amplification (col. 21, lines 22-25).

Regarding claim 31, Lizardi teaches that primers may include modified nucleotides to make them exonuclease-resistant (col. 10, lines 24-28; col. 13, lines 27-31). Therefore Lizardi teaches exonuclease activity.

Regarding claim 33, Lizardi teaches adding exonuclease to digest unligated circles (col. 10, lines 28-33; col. 24, lines 41-61).

Regarding claim 35, Lizardi teaches that modified nucleotides are used in the amplification (col. 21, lines 22-25).

Regarding claims 36 and 37, Lizardi teaches oligonucleotides attached to solid support, including glass (col. 14, lines 34-43, 65-67; col. 15, lines 1-10).

Regarding claims 38 and 39, Lizardi teaches primers which include modified nucleotides to make them exonuclease-resistant (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claims 44, 45 and 47, Lizardi teaches that phosphorothioate nucleotides are positioned at the 5'-end of the primer to make it exonuclease-resistant (col. 10, lines 24-28; col. 13, lines 27-31). Therefore Lizardi anticipates the limitations of an exonuclease-resistant primer containing at least one nucleotide which makes it resistant to exonuclease activity, a modified nucleotide and a phosphorothioate nucleotide.

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Regarding claim 48, Lizardi teaches three or four phosphorothioate nucleotides (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claim 49, Lizardi teaches the phosphorothioate nucleotides being at the 5' end of the primer (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claims 51 and 52, Lizardi teaches the following DNA polymerases to be used: bacteriophage ϕ 29 DNA polymerase, phage M2 DNA polymerase, VENT DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, PRD1 DNA polymerase, T4 DNA polymerase holoenzyme (col. 17, lines 66-67, col. 18, lines 1-11). Therefore, since the claim language links 3', 5'-exonuclease activity with these enzymes, and Lizardi specifically teaches them, Lizardi inherently teaches polymerases with 3' \rightarrow 5' exonuclease activity.

Regarding claim 53, Lizardi teaches bacteriophage ϕ 29 DNA polymerase (col. 17, lines 66-67, col. 18, lines 1-11) and exonuclease-resistant primers (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claims 55 and 56, Lizardi teaches the Tfl DNA polymerase which does not exhibit 3'-5' exonuclease activity (col. 37, lines 52-54).

Regarding claim 69, Lizardi teaches isothermal amplification conditions (col. 5, line 8; col. 36, lines 48-56).

Regarding claim 70, Lizardi teaches using secondary and tertiary primers with sequences complementary to the ATC, with secondary and tertiary primers not being complementary to the initial primer or to each other (col. 25, lines 36-49). The SDCA can be performed simultaneously with RCA, resulting in exponential amplification (col. 25, lines 50-57; col. 28, lines 8-18).

Therefore, since initial primers and the secondary and tertiary primers anneal to different parts of ATCs and are present simultaneously in the reaction mixture, Lizardi teaches simultaneous hybridization of primers to the ATCs.

B) Lizardi does not teach random primers, nucleotides which confer nuclease resistance to an amplification product, DNA with unknown sequence, dNTPs being phosphorothioate nucleotides, modified nucleotide being a 3'-terminal nucleotide or a DNA polymerase which does not exhibit 3' → 5' exonuclease activity.

C) Regarding claim 1, Landers et al. teach generation of reduced complexity genomes by amplification of genomic double-stranded DNA circles (YACs) with multiple arbitrary (= random) primers (col. 17, lines 28-42 and 60-64).

Regarding claims 8 and 9, Landers et al. teach that the sequence of the random primers contains the N_x residues of the DOP-PCR primers (col. 17, lines 35-39). Landers et al. teach DOP-PCR primers containing x N residues, where x is an integer from 0 to 9, therefore Landers et al. teach hexamers and octamers.

Regarding claim 25, Landers et al. teach amplification of unknown sequences (col. 17, lines 31-34).

D) Regarding claim 1, Navarro et al. teach amplification of circular RNA viroids using multiple random hexamers and AMV reverse transcriptase (Fig. 1; page 59, first paragraph; page 60, paragraphs 4 and 5; page 61, first paragraph).

Regarding claim 14, Navarro et al. teach amplification of single-stranded RNA circles (Fig. 1).

Regarding claim 57, Navarro et al. teach reverse transcriptase (Fig. 1; page 60, paragraphs 4 and 5).

Regarding claim 58, Navarro et al. teach reverse transcriptase and amplification of single-stranded RNA circles (Fig. 1; page 60, paragraphs 4 and 5).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have included random primers of Landers et al. in the method of Lizardi. The motivation to do so, provided by Landers et al., would have been that random primers allowed for amplification of unknown DNA sequences (col. 17, lines 31-34). It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the amplification of RNA circles by random primers of Navarro et al. in the method of Lizardi and Landers et al. The motivation to do so, provided by Navarro et al., would have been that amplification of circular pathogenic RNA provided means of cloning the RNAs from small amounts of sample with unknown sequence (page 60, second paragraph).

E) Neither Landers et al. nor Navarro et al. teach nucleotides which confer nuclease resistance to an amplification product, dNTPs being phosphorothioate nucleotides or a modified nucleotide being a 3'-terminal nucleotide.

F) Regarding claims 1 and 29, Eckstein et al. teach that deoxynucleoside 5'-O-(1-thiotriphosphates), or phosphorothioates, are substrates for DNA and RNA polymerases (Abstract; page 97, first paragraph).

Regarding claim 41, Eckstein et al. teach exonuclease III with 3',5'-exonuclease activity (page 97, fourth paragraph).

Regarding claims 46 and 47, Eckstein et al. teach that incorporation of single phosphorothioate group at the 3' end of a DNA strand prevents its degradation by exonuclease III, an enzyme with 3',5' activity (page 97, fourth paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used phosphorothioate dNTPs of Eckstein et al. in the amplification method of Lizardi, Landers et al. and Navarro et al. The motivation to do so, provided by Eckstein et al.,

would have been that phosphorothioate containing DNA was resistant to degradation by nucleases and the sulfur atom conferred many favorable chemical properties (Abstract).

8. Claims 32, 42 and 59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lizardi (U.S. Patent No. 5,854,033; cited in the previous office action), Landers et al. (U.S. Patent No. 6,703,228; cited in the previous office action), Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action) and Eckstein et al. (Trends in Bioch. Sci., vol. 14(3), pp. 97-100, 1989; cited in the previous office action) as applied to claims 1, 31 and 38 above, and further in view of Skerra (Nucleic Acids Research, Vol. 20, pp. 3551-3554, 1992; cited in the previous office action).

A) Claims 32 and 42 are drawn to a polymerase with 3'→5' exonuclease activity and claim 59 to the use of a mixture of primers sensitive to and resistant to exonuclease activity.

B) Lizardi, Landers et al. and Eckstein et al. do not teach primers resistant to 3'→5' exonuclease activity, the resistance being conferred by a phosphorothioate nucleotide at the 3'-end of the primer or the use of a mixture of exonuclease-sensitive and exonuclease-resistant primers in the amplification reaction.

C) Skerra teaches that incorporation of a phosphorothioate nucleotide at the 3'-end of the primer renders it inactive to the 3'→5' exonuclease activity of DNA polymerases such as Vent and Pfu. The reference also teaches use of a mixture of exonuclease-sensitive and exonuclease-resistant primers in the amplification reaction (Abstract; page 3553; Fig. 2).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used primers of Skerra with phosphorothioate nucleotides at the 3'-end in the amplification method of Lizardi, Landers et al., Navarro et al. and Eckstein et al. The motivation to do so, provided by Skerra, would have been that the 3'-end phosphorothioate nucleotide rendered

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the primers resistant to 3' -> 5' exonuclease activity of the polymerase used in the reaction, resulting in an improved yield of the amplification product (page 3553, third paragraph).

9. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E. Strzelecka whose telephone number is (571) 272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

October 25, 2005

TERESA STRZELECKA
PATENT EXAMINER

Teresa Strzelecka